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DETERMINATION OF PARAQUAT IN SUNFLOWER SEEDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The herbicide paraquat was determined with extracts from 1-g samples of sunflower seeds. The liquid chromatography procedure utilized a microparticle (10 μm) C_{18} reversed-phase column and isocratic elution with 27% acetonitrile in water, 10 m*M* in the sodium salt of octanesulfonic acid. Eluted paraquat was detected at 254 and 280 nm and quantitated by paraquat internal standard peak height ratios. This procedure provided linear working curves over the concentration range of 0–20 μg/g of paraquat. Recovery of paraquat varied from 89–101%, with an average recovery of 93%. Good agreement was obtained in the comparison of results of the described procedure with those from a well established UV procedure.

INTRODUCTION

Paraquat is widely used in agriculture as a contact herbicide for weed control in untilled areas and in sugarcane fields; it is also used as a desiccant which aids in the harvesting of mature sunflower seeds[1]. Because of the increase in the use of sunflower seeds as a food and for producing oil, stringent guidelines have been established for tolerance levels of paraquat residues in seeds to be consumed as an edible crop. The levels currently allowed are approximately 5 ppm in the outer hull portion and 2 ppm in the edible kernel portion[2].

Most of the methods now used for determining paraquat levels in plant material have one or more shortcomings. An excellent review of available methods for determining paraquat has recently been published[3]. Generally speaking, spectrophotometric methods are time consuming and are not amenable to the use of an internal standard[4]; thin-layer methods produce results that are difficult to quantify[5,6]; and gas chromatographic methods require reduction of paraquat to a volatile product[7]. High-performance liquid chromatography (HPLC) offers a nearly ideal method for determining ionic substances with limitations only in the selectivity and sensitivity of existing detectors. With HPLC two distinct modes are available for the separation of ionic species such as paraquat: ion-exchange and ion-pair liquid

chromatography. An ion-exchange method for determining paraquat in urine has been described in recent literature[8]. Because of the high degree of reproducibility of reversed-phase columns used in ion-paired modes and because this type of chromatography has been used successfully in determining paraquat in marijuana[9], reversed-phase ion-pair liquid chromatography was selected for use in this determination. A discussion of ion-pair chromatography is beyond the scope of this paper; an excellent detailed review has been published describing this mode of chromatography[10].

The method to be described is rapid and sensitive and permits the use of relatively small (0.5–1.0 g) samples. The diethyl homolog of paraquat was used as an internal standard, and was carried through all procedural steps to compensate for physical and chemical losses of paraquat. The results of this procedure agree closely with those obtained by a well-established UV procedure[4].

MATERIALS AND METHODS

*Materials**

The chromatographic system used for this study has been described elsewhere[9]. The C_{18} SEP-PAKs were obtained from Waters Assoc., Milford, Mass. U.S.A.

Mobile phase components were "Nanograde" acetonitrile (Mallinckrodt, St. Louis, Mo., U.S.A.), water purified by a MILLI-Q system (Millipore Corp., Bedford, Mass., U.S.A.), reagent grade sulfuric acid (Fisher Scientific, Pittsburgh, Pa., U.S.A.), and the sodium salt of octanesulfonic acid (Eastman-Kodak, Rochester, N.Y., U.S.A.) used without further purification. Paraquat was obtained from Chevron (Richmond, Calif., U.S.A.); the diethyl homolog of paraquat was obtained from ICI (Macclesfield, Great Britain), hydrochloric acid, sodium hydroxide, methanol, isopropanol, and chloroform were analytical-reagent grade.

Procedure

A procedure previously used for determining paraquat in marijuana[9] was modified for use in this study. Home grown sunflower seeds that were known to be uncontaminated with paraquat were used as control materials. These seeds were used to prepare calibration standards by spiking with known quantities of paraquat, and then carrying the spiked materials through the procedure. Since different levels are specified as tolerance limits for the outer (hull) and edible (kernel) portion of the seeds, separate sets of standards were prepared from these two materials. Both hulls and kernels from uncontaminated seeds were spiked in the range 0–20 μg/g (ppm) in paraquat, with 200 μg/g of the diethyl homolog, added as 2.00 ml of 100 ppm of the iodide salt to each sample as internal standard.

Approximately 1 g of sunflower seed hulls or kernels was weighed out from seeds dried for 4–6 h at 100°, and internal standard was added to the sample as an aqueous solution. A 10-ml volume of 6 *M* HCl was added, and the material was sonified for 10 min, vortexed to mix thoroughly, and again sonified for 10 min. The

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

material was filtered through glass fiber filter paper (Whatman GF/A), and evaporated under vacuum to dryness. A 4-ml solution of 0.10 *M* phosphate buffer, pH 7.00, was added to dissolve the residue and exactly 1.00 ml of the resulting solution was passed through a C_{18} SEP-PAK™ which had been prepared for use according to the manufacturer's directions. After the 1.00 ml of buffer solution was passed through the SEP-PAK for cleanup, and the eluate was collected, the SEP-PAK was washed with 3.00 ml of water. The water wash was collected and combined with the original eluate, and mixed thoroughly. Then, 25 μl of the combined eluates were injected into the chromatograph. The mobile phase used was acetonitrile–water (27:73), adjusted to pH 3.00 with sulfuric acid, 10 m*M* in sodium octanesulfonate. A μBondapak C_{18} (Waters Assoc.) reversed-phase column was used for separation. Detection was at 254 and 280 nm, both wavelengths being followed with a dual-pen recorder (Houston Instruments). Flow-rate throughout was held at 1.5 ml/min.

RESULTS

The calibration curve obtained for sunflower seed hull samples spiked with paraquat is shown in Fig. 1. Ratios of peak heights of paraquat to peak heights of the diethyl homolog internal standard were observed to be linear over the range of standards used. Recoveries were calculated by comparing peak heights obtained from volumetric dilutions of equivalent amounts of paraquat. Recoveries were in the range of 89–101%, with an average recovery of 93%. Calibration curves prepared from the edible (kernel) portion were less than satisfactory, with widely scattered results. This is attributed to the presence of interfering material(s) in the edible material which co-elutes with paraquat under the described chromatographic conditions. The extent of this interference is shown in Fig. 2, in which the lower trace shows the presence of an interfering peak at the same retention time as paraquat. A comparison with Fig. 3 illustrates the absence of interference at the retention time

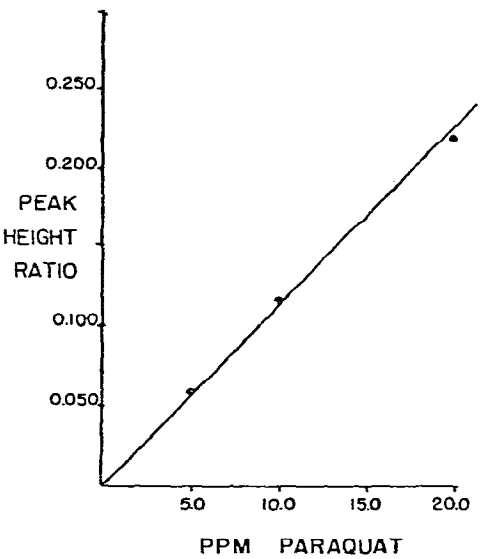

Fig. 1. Calibration curve for described method.

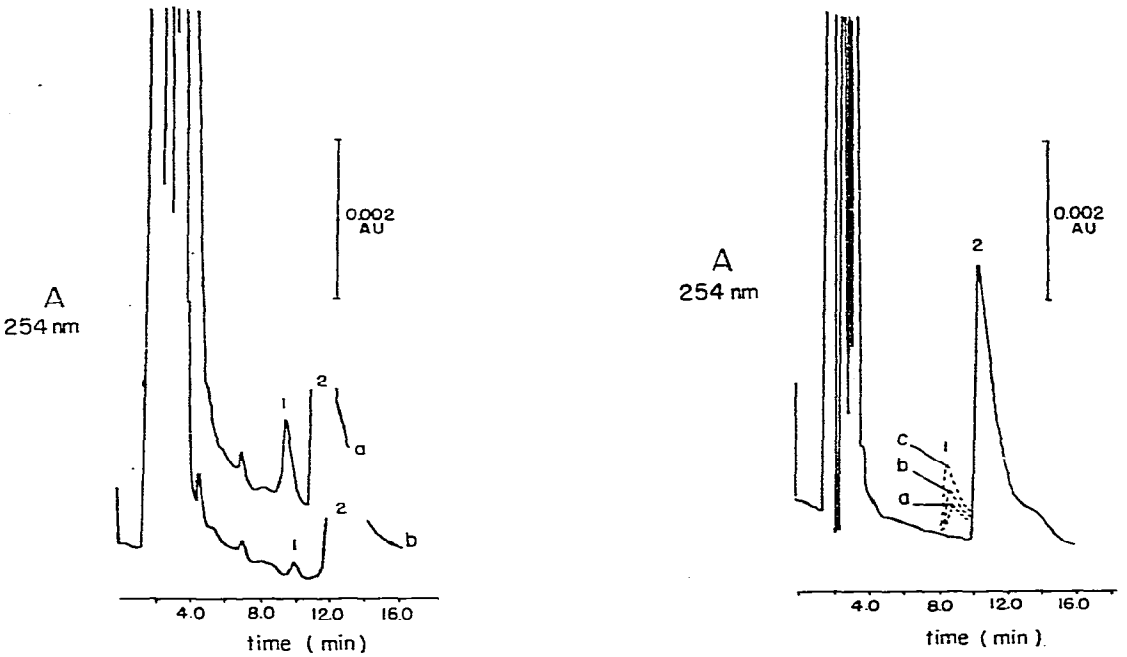

Fig. 2. Sunflower seed hull extract (spiked to 23 ppm paraquat) (a) and kernel extract (b). Peaks: (a), 1 = paraquat; 2 = diethyl homolog internal standard; (b), 1 = interfering substance from edible portion; 2 = diethyl homolog internal standard. Conditions as in the *Procedure* section.

Fig. 3. Chromatogram of 0 ppm (solid line), 10 ppm (1a); 15 ppm (1b), and 20 ppm (1c) spiked hull samples. Peak 2: diethyl homolog internal standard. Conditions as in the *Procedure* section.

of paraquat in the hull samples. Attempts to remove the interference in the kernel samples by changing chromatographic conditions were not successful.

Interferences from other herbicides commonly used with paraquat were investigated by comparing retention times of potentially interfering materials with those of paraquat. As can be seen from Table I, most of the compounds investigated have relative retention times sufficiently different from paraquat to avoid interference. Both cyanazine and diquat are potential interferences under the described experimental

TABLE I

CHROMATOGRAPHY INTERFERENCE STUDY OF OTHER HERBICIDES (100 ppm)

Compound	*Relative retention (paraquat = 1.00)*	*Compound*	*Relative retention (paraquat = 1.00)*
Alachlor	1.056	2,4-D (acid)	1.970
Amiben	0.604	Ethyl Paraquat	1.250
Atrazine	1.480	Linuron	4.300
Carbaryl	1.566	Malathion	1:85
CDEC	10.97	Methyl Parathion	5.77
Cyanazine	0.962	Monolinuron	1.736
Diazinon	9.98	Simazine	0.858
Diquat	0.950	Trifluralin	0.740
Diuron	2.190	2,4,5-T (acid)	3.790

TABLE II

RESULTS OF PARAQUAT DETERMINATION ON HULLS OF SUNFLOWER SEEDS FROM BRUNSWICK, Ga., U.S.A.

Sample number	*Paraquat concentration (μg/g)*
1	1.4_7
2	2.7_8
3	2.1_2
4	2.1_0
5	1.6_3
6	2.9_3
7	2.7_5
8	1.9_1

to the paraquat peak. This low resolution can be improved by slowing the flow-rate to 1.0 ml/min, under which condition diquat and cyanaine are sufficiently resolved from paraquat to allow quantitation.

This method was applied to authentic samples of sunflower seeds which had been desiccated with paraquat before they were harvested. These samples were collected from Brunswick, Ga., U.S.A., in our investigation of suspected paraquat in respirable dust caused by mechanical handling of the seeds. The samples were dried, hulled, and the hulls were analyzed by the described procedure. The results are given in Table II. As can be seen, substantial amounts of paraquat remained on the outer portions of the seeds. In order to verify our results and estimate their accuracy, the same hulls were also analyzed by a well established spectrophotometric procedure[4]. The results obtained with the two methods agreed closely (Table III).

TABLE III

COMPARISON OF UV AND HPLC PROCEDURES

Sample No.	*UV (μg/g)**	*HPLC (μg/g)**
1	3.0	2.7_8
2	2.4	2.9_3
3	2.7_2	2.7_5

* Average of three values.

DISCUSSION

This procedure is a useful approach to the determination of paraquat in plant materials. The described cleanup procedure with a C_{18} SEP-PAK is simple and rapid, and avoids the time consuming and tedious solvent extraction steps originally used with the procedure.

It is very important to "pre-condition" the reversed-phase column with a large volume of the mobile phase before use. Up to 1–2 l of mobile phase may need to be passed through the C_{18} column before resolution and retention is observed for paraquat and its diethyl homolog. The amount of pre-conditioning needed varies with the prior use of the column to be conditioned. Typically, columns that have been used extensively require more pre-conditioning. In this connection, passage of mobile

phase through the column at 0.2–0.3 ml/min overnight (12 h) has been shown to be effective for new columns. This process can be continued as necessary with older columns. It is suggested that the progress of column conditioning be monitored by injection of aqueous standards containing both paraquat and the diethyl homolog internal standard. When adequate resolution and retention are reached, the column may then be used as described. Once conditioned, the column will perform with adequate resolution for 2–3 months use, based on separation of 30–35 samples per day.

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